

# The Morphogenetic Code and Colon Cancer Development

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The initiating genetic lesion in sporadically occurring cancers is impossible to identify. The existence of rare inherited cancer syndromes has helped to uncover some of the mutations that can initiate tumorigenesis. Most of these initiating lesions affect genes belonging to morphogenetic signaling pathways. We review the evidence that the cellular fate of individual epithelial cells in the adult is nonautonomous and depends on extrinsic information, just like cells in a developing embryo. Cancer stem cells need to disrupt these extrinsic restraints to gain an autonomous clonal proliferative advantage over neighboring stem cells.

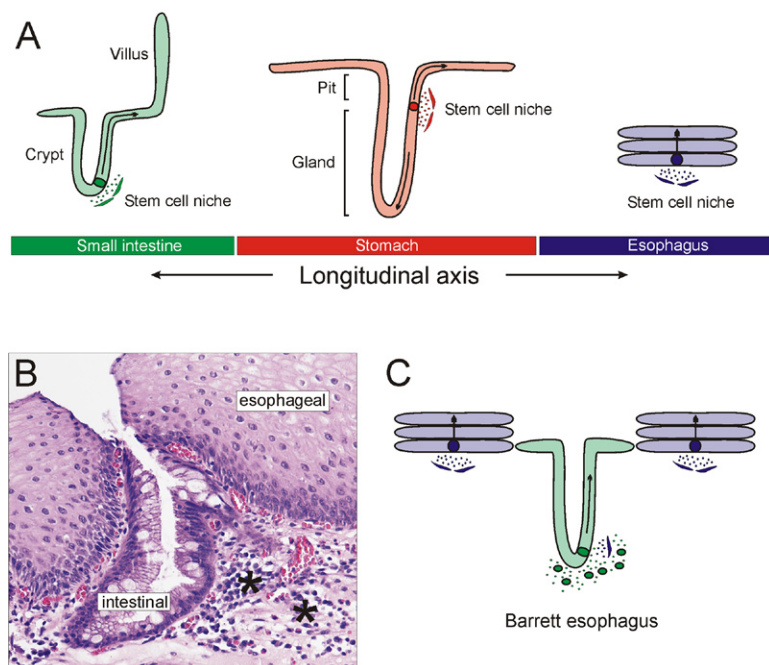
Multicellular organisms are built from subsets of specialized cell types that are allocated to different organs in a predefined location and quantity. To develop and maintain such a level of complexity, individual cells are subject to stringent conditions that determine when and where they live and what they do (i.e., cell fate). It is therefore a hallmark of cells in multicellular organisms that cell fate is not regulated at the level of the individual cell but at the population level by extracellular signals. As a result, it is not surprising that mutations that disturb this type of intercellular signaling have now been established as important initiators of the genetic cancer cascade in epithelial tissues. Our current understanding of the histogenesis of a colon carcinoma suggests that this process starts with a genetically mutated cancer stem cell that gains a competitive advantage in proliferative capacity over neighboring stem cells. To understand how a colonic stem cell is able to gain such a competitive advantage, it is first important to recognize that cellular fate is controlled by environmental cues in the intestine.

## Evidence for Position-Dependent Cell Fate in the Adult

The fate of a cell in a developing embryo is determined by its position. The mechanisms used to communicate positional information during development have been the subject of experimental biology for more than a century (Wolpert, 1996). It is much less appreciated that there is important evidence for a similar mechanism of cell-fate regulation in the adult, especially in rapidly dividing tissues. Similar to that of cells in a developing organ, the cellular fate of epithelial cells in the adult gut is regulated by extrinsic signals (extracellular information that is nonautonomous to the cell). This information patterns epithelial-cell fate along two important axes that are addressed separately below.

## The Vertical Axis

Each stem cell in the gastrointestinal tract generates a variety of more committed precursor cells that undergo a transient phase of cellular proliferation. Each descendant of these precursor cells exits the cell cycle, is allocated to a specific cell lineage, and undergoes a process of maturation into a differentiated epithelial cell that lives a short life before it dies. This cycle of life and death is a linear process from the stem-cell niche toward the compartment of differentiated cells. A cell that migrates along this vertical axis has to make important decisions regarding its cell-cycle status, lineage commitment, maturation status, and death. In a hallmark study by the Gordon laboratory, it was shown that the fate of epithelial cells is regulated in a non-autonomous way (Hermiston et al., 1996). This group has made use of the fact that each stem cell along the longitudinal axis of the gut produces its offspring as monoclonal populations. The authors produced a series of chimeric mice in which two fertilized zygotes (one wild-type and the other transgenic) or one (transgenic) embryonic stem cell and a fertilized zygote are fused and the clonal offspring of normal and genetically engineered cells can be juxtaposed. In one such chimeric mouse, the cell-cell adhesion molecule E-cadherin was specifically overexpressed in maturing small-intestinal epithelial cells derived of E-cadherin transgenic 129/SV mouse ES cells, whereas cells derived from the wild-type C57Bl/6 blastocyst were normal. As a result, E-cadherin-overexpressing cells produced by transgenic stem cells were migrating side by side with normal epithelial cells on a single villus. Overexpression of E-cadherin considerably slowed migration of transgenic cells. Even though the speed of migration of transgenic cells was much reduced, their maturation status along the vertical axis was completely position



**Figure 1. Cell-Fate Specification along the Longitudinal Axis Is Determined by a Stem-Cell Niche**

(A) Bone-marrow-derived stem cells have the capability to generate a module of cell types that is typical for a given location along the longitudinal axis of the gut. This illustrates the presence of extrinsic signals from the mesenchyme specifying cell fate along the longitudinal axis at the level of the stem-cell niche. Here these mesenchymal cells are schematically depicted as secreting a mix of extracellular factors specific for that particular location along the longitudinal axis.

(B) In areas of metaplasia, cell types are generated that are not appropriate for that specific location along the longitudinal axis. In Barrett's esophagus (B), the esophageal multilayered squamous epithelium is replaced by intestinal-type columnar cell epithelium (note presence of goblet cells in the setting of chronic inflammation (asterisks indicate inflammatory infiltrate)).

(C) Such a phenotypic switch in cell-type generation could conceivably be caused by the influence of the inflammatory infiltrate on the composition of extracellular signals in the stem-cell niche.

dependent and the same as their normal counter parts. This experiment nicely demonstrated that the intestinal epithelial life cycle is extrinsically regulated.

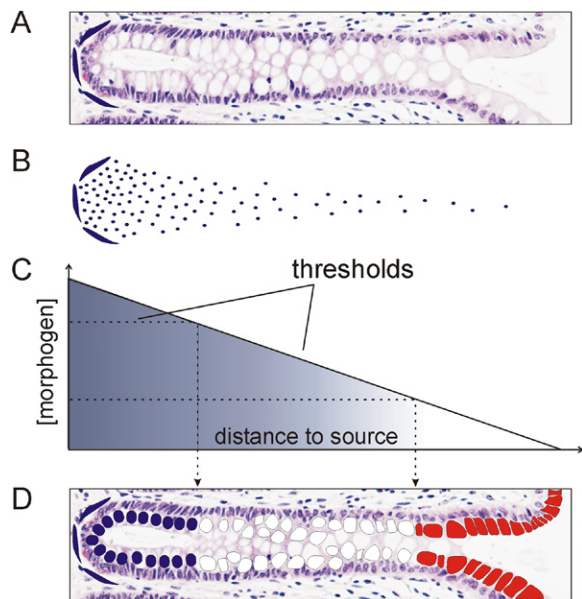
### The Longitudinal Axis

Cell fate is not only coupled to the topography of the epithelial life cycle but also dependent on cellular position along a proximal-to-distal axis of the gut tube. Regulation along this second axis ensures that precursor cells generate a module of specialized cell types that suits that particular region of the gut. Cell-fate regulation along the longitudinal axis is a topic that is less well investigated in the adult, especially when compared to our understanding of the regulation along the vertical axis. Two lines of evidence suggest, however, that cell-fate specification along the longitudinal axis may require continuous patterning by epithelial-cell nonautonomous information similar to the vertical axis.

### Plasticity of Stem Cells

The ongoing nature of precursor-cell programming along the longitudinal axis and its dependence on extrinsic factors may be best exemplified by recent findings in patients and animals receiving bone-marrow transplants. Bone-marrow transplants contain stem cells that are able to produce cell lineages specific for many of the recipient's tissues. Bone-marrow stem cells are able to colonize small-intestinal crypts in a clonal manner that is typical for gut stem cells and stably populate adjacent villi with cells of all intestinal epithelial-cellular lineages that appear histologically normal (Jiang et al., 2002; Rizvi et al., 2006). The interpretation of some of the work performed with bone-marrow-derived stem cells remains controver-

sial, and it is still debated whether stem-cell recruitment and transdifferentiation really occurs, since fusion of recruited bone-marrow-derived stem cells with resident stem cells would be an alternative scenario (Marx, 2004; Pauwelyn and Verfaillie, 2006). The distinction between fusion and transdifferentiation is an important one because a recruited stem cell that fuses with a resident stem cell would acquire information that is intrinsic to the resident stem cell, a scenario that does not support a nonautonomous control of patterning along the longitudinal axis (although it would not necessarily argue against it). In recent experiments in irradiated mice, most of the bone-marrow-derived stem cells that contributed to intestinal epithelial tissue seemed to do so through fusion with resident stem cells (Rizvi et al., 2006). However, in a study by Houghton et al. (2004) in the *Helicobacter*-infected stomach, it was shown that when female mice were transplanted with green fluorescent protein (GFP)-expressing male bone-marrow donor cells the donor-derived epithelial cells (GFP-positive, negative for hematopoietic marker CD45, positive for epithelial marker cytokeratin) contained single X and Y chromosomes, indicating that they truly transdifferentiated without cell fusion upon recruitment. The fact that such recruitment and transdifferentiation can occur (however rare it may be) is a proof of principle and a strong argument per se in favor of the existence of a stem-cell niche in adult epithelial tissues. It would mean that the niche contains signals that are nonautonomous to the stem cell and inform and guide the recruited stem cell to ensure the generation of the appropriate cell lineage depending on its position along the longitudinal axis (Figure 1A). More evidence is needed to further clarify this notion.



**Figure 2. The French Flag Model Applied to Cell-Fate Regulation in the Colonic Crypt**

When a morphogen is produced at one end of the crypt, for example, by the mesenchymal cells around the crypt base, as depicted schematically in (A), a concentration gradient will form along the axis of renewal (B). The French flag model (Wolpert, 1996) is used to depict the concentration-dependent mechanism of action of a morphogen. In this model, a morphogen can cause three distinct responses in its target cells, each at its own threshold concentration. The different responses are indicated in three different colors: blue, white, and red (C). In this way the phenotype of a cell is determined by its precise position in this morphogen concentration gradient, and in this model, therefore, by its distance from the base of the crypt (D).

### Metaplasia

The second example of the dynamic nature of precursor-cell programming along the longitudinal axis is the existence of metaplasias (Slack, 1986; Tosh and Slack, 2002). Epithelium in areas of inflammation can change into a phenotype that is inappropriate for the location along the longitudinal axis. Such change is known as metaplasia and is easily recognized histologically by the presence of a cell population that is not normally present in the original tissue (e.g., intestinal goblet cells in Barrett's esophagus; Figure 1). Examples in the gastrointestinal tract include epithelial columnar cell and/or intestinal metaplasia of the esophagus in Barrett's esophagus (Figures 1B and 1C), intestinal metaplasia and pancreatic metaplasia of the stomach in atrophic gastritis, gastric mucin cell metaplasia of the duodenum in peptic duodenitis, pseudopyloric metaplasia of the small intestine in Crohn's disease, and Paneth cell metaplasia of the colon in inflammatory bowel disease. It has been suggested that metaplasias might result from somatic DNA mutations. However, a monoclonal proliferation of metaplastic cells would be expected if metaplasia were due to a somatic mutation and metaplasias were multifocal lesions (Thompson et al., 1983) that were polyclonal in nature (Nomura et al., 1998). An epigenetic mechanism for the development of metapla-

σίας is therefore more likely. We would hypothesize that differentiation of gut precursor cells is most likely determined by extracellular signals that are altered in the above situations of inflammation due to factors that alter the microenvironment of the precursor-cell niche (Figure 1C). Thus, substantial evidence indicates that extrinsic signals generate the spatial information that is necessary for the generation of the appropriate cell-type repertoire by stem cells along the longitudinal axis and the subsequent maturation and cell death of their descendants along the axis of renewal.

### Extrinsic Regulators of Cell Fate

Since cellular renewal in the adult gut is regulated by extrinsic information (information that is nonautonomous to the individual epithelial cell) along two different axes, it has important similarities with patterning events in the developing embryo. A small number of evolutionarily conserved signaling pathways have emerged in developmental biology as the master regulators of position-dependent cell fate. Most of these master regulators are morphogens, molecules that act by forming concentration gradients through a tissue (Figure 2) (Lawrence and Struhl, 1996). Although most morphogens are proteins, this does not mean that concentration gradients necessarily arise by passive diffusion. In fact, proteins of the Hedgehog and WNT families of morphogens are lipid modified and very poorly soluble (Eaton, 2006). Many different levels of active regulation of gradient formation probably exist, depending on the morphogen, organism, and tissue (Torroja et al., 2005; Vincent and Dubois, 2002; Zhu and Scott, 2004). Morphogens have a concentration-dependent effect on their target cells; as a result, the distance of the target cell from the source of the morphogen determines its phenotype. The WNT, Hedgehog (Hh), tumor growth factor- $\beta$  (TGF- $\beta$ ), and several receptor tyrosine kinase families are distinct morphogenetic pathways that have been conserved from fruit fly to man. This protein family shares a highly similar intracellular tyrosine kinase signaling pathway through the RAS-RAF-Mitogen-Activated Protein Kinase and PI3 kinase-AKT signaling pathways (Eswarakumar et al., 2005; Schlessinger, 2000). The family includes the fibroblast growth factor (FGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) tyrosine kinase receptors. Receptor tyrosine kinases share a common signaling pathway and are perhaps better known to many as growth factors in the adult organism, but all families listed above also have important roles as morphogens during development (Bottcher and Niehrs, 2005; Coultas et al., 2005; Hoch and Soriano, 2003; Shilo, 2005). The members of this small number of protein families act in varying constellations to form what has previously been termed a "morphogenetic code" (Hogan, 1999). The morphogenetic code encodes information that enables communicating groups of signaling centers to execute basic programs such as the formation of a field of pro-

genitor cells into, for example, a bud or a gland. Although we still have limited information on the role of morphogens in the maintenance of homeostasis in the normal adult colonic epithelium, the first examples of the role of such molecules are slowly starting to emerge.

For example, transcriptional activity of WNT- $\beta$ -catenin signaling acts to maintain a precursor-cell phenotype in intestinal epithelial cells (Gregorieff and Clevers, 2005; van de Wetering et al., 2002). As colonic epithelial cells move up the crypt toward the luminal surface, they start to produce the Hh family member Indian Hedgehog, which regulates their differentiation (van den Brink et al., 2004). Members of the TGF $\beta$  family regulate colonic epithelial apoptosis in vivo (Dunker et al., 2002; Hardwick et al., 2004). Of the FGF family, Keratinocyte Growth factor (KGF/FGF-7) is expressed by myofibroblast-like cells that underlie the colonic enterocytes, and its receptor (a splice variant of FGFR2) is expressed on epithelial cells (Finch et al., 1996). KGF acts as a positive regulator of precursor-cell proliferation and the goblet cell lineage (Housley et al., 1994). The EGF family members transforming growth factor- $\alpha$  and Amphiregulin are expressed in superficial colonic enterocytes (Johnson et al., 1992; Thomas et al., 1992) and probably act in a paracrine manner to stimulate colonic epithelial precursor-cell proliferation (Park et al., 1997). The importance of these morphogenetic pathways in the maintenance of epithelial homeostasis is underscored by the accumulating evidence that mutations that disrupt them are the foremost *initiators* of the genetic cancer cascade identified to date.

### Initiators of Cancer Development

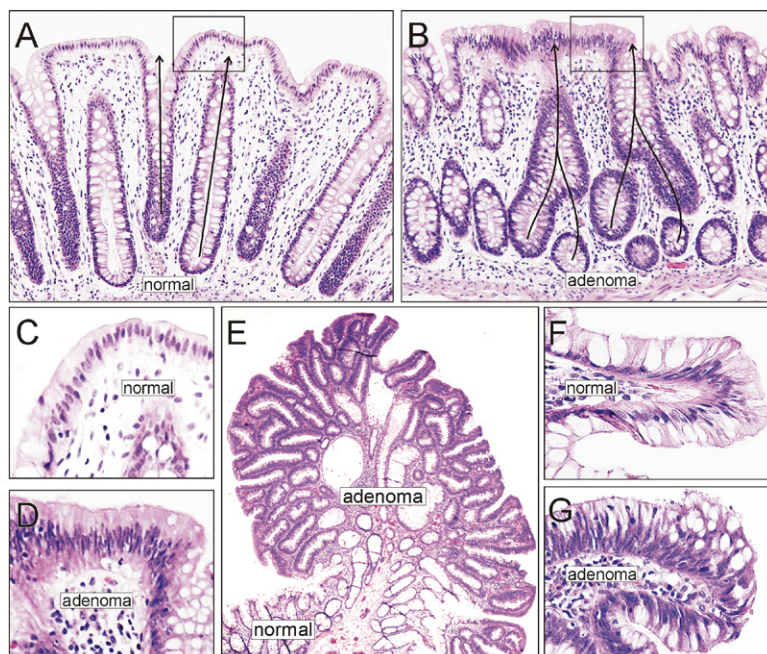
Although a massive research effort has identified many genes that are mutated in cancer development, we only know relatively few mutations that *initiate* this process. Some groups have been able to identify a few of these cancer initiators through the study of families with rare inherited cancer syndromes. Generally, affected members of these families are heterozygous for the inherited mutated gene and develop cancer from a stem cell that is affected by a somatic mutation in the remaining wild-type allele. Many of the inherited mutations in cancer syndromes have now been identified. Some of these mutations affect stability genes, such as mutations in DNA mismatch repair genes that have been found in hereditary nonpolyposis colorectal cancer (HNPCC) (Chung and Rustgi, 1995; Vogelstein and Kinzler, 2004). However, a second class of initiating mutations that has emerged from these families involves mutations in pathways that form part of the morphogenetic code. Mutations in at least three distinct morphogenetic signaling pathways cause gastrointestinal polyposis syndromes. First, mutations in the *adenomatous polyposis coli* (*APC*) gene, a critical component of the WNT pathway that acts to restrict its activity cause familial adenomatous polyposis (FAP) syndrome (Kinzler et al., 1991; Nishisho et al., 1991). Second, mutations that disrupt expression of

the *BMP receptor 1A* and TGF $\beta$  family signaling mediators *SMAD4* and *endoglin* have been found in juvenile polyposis syndrome (JPS) (Howe et al., 1998, 2001; Sweet et al., 2005). Although both SMAD4 and Endoglin are shared by the TGF $\beta$  and BMP signaling pathway, this does not mean that disruption of TGF $\beta$  signaling per se can initiate colorectal carcinogenesis. In mice with conditional inactivation of the TGF $\beta$  receptor type II (a mutation often found in microsatellite unstable colon cancers), no histological abnormalities were found in the colon, the mice had a rate of colonic epithelial proliferation that was comparable to controls, and no spontaneous polyposis developed (Biswas et al., 2004). The authors showed in the same paper that the conditional TGF $\beta$  RII mutation did substantially enhance mutagen-induced carcinogenesis, more consistent with a role for TGF $\beta$  pathway mutations in tumor progression. Interestingly it was recently shown in mice that specific ablation of Smad4 expression in T cells resulted in a JPS phenotype, whereas disruption of Smad4 expression from epithelial cells did not (Kim et al., 2006). This could represent an important example of the importance of the stroma in epithelial cancer initiation. However, the promoters that were used to drive the epithelial-specific expression of the Cre transgene and a dominant-negative form of Smad4 are poorly characterized with regard to their use in the intestinal epithelium, and it is not certain that they target intestinal epithelial stem cells, so these results await further confirmation.

The third example is that mutations that affect receptor tyrosine kinase signaling pathways have been found in inherited polyposis syndromes. Mutations in *PTEN*, a phosphatase that antagonizes PI3 kinase activity, cause Cowden's syndrome, a syndrome with hamartomas in the gastrointestinal tract, central nervous system, and skin, and tumors of the breast and thyroid gland (Liaw et al., 1997; Marsh et al., 1998). The function of *LKB1*, the gene mutated in Peutz-Jeghers syndrome (Hemminki et al., 1998), another important gastrointestinal polyposis syndrome, was recently potentially linked to the PI3 kinase pathway when it was shown that it acts to negatively regulate mammalian target of rapamycin (mTOR), a downstream effector of PI3 kinase-AKT signaling (Corradetti et al., 2004; Shaw et al., 2004). No germline mutations have been identified in the RAS-RAF-MAPK pathway, the other major pathway that is associated with receptor tyrosine kinase signaling. Mutations in both *RAS* and *RAF* are found in very early lesions however (Alrawi et al., 2006; Beach et al., 2005; Goldstein, 2006; Takayama et al., 2001; Yamashita et al., 1995), and evidence in mice supports the concept that *RAS* mutations can initiate tumorigenesis (Janssen et al., 2002). It is important to note that, although receptor tyrosine kinases are the classical activators of the RAS-RAF-MAPK and PI3 kinase pathways, they can also be activated by other extracellular signals.

Although all of these syndromes are rare diseases, they represent at the same time some of the rare cases





**Figure 3. Small Adenomas Expand by Crypt Fission**

Small adenomas expand by crypt fission. (A) Epithelial migration in normal crypts is in a straight line from the precursor-cell compartment toward the luminal surface of the gut. (B) A microadenoma consisting of two crypts that are both in the process of fissioning. The two different crypt bases are still in conjunction with a single crypt lumen at the top. Normal epithelium consists of a polarized monolayer of well-differentiated cells (C), whereas the epithelium of the microadenoma (D) shows crowding of the cells with enlarged hyperchromatic nuclei.

(E–G) Histology of a larger adenomatous polyp from a patient with familial adenomatous polyposis. This classical adenoma is a mucosal lesion consisting of hyperproliferative dysplastic epithelium that grows as a protruding mass into the lumen of the colon (E). The superficial epithelium of the adenoma (G) shows the typical features of dysplasia, such as cellular crowding and loss of epithelial polarity, whereas the adjacent normal epithelium is a highly polarized monolayer (F).

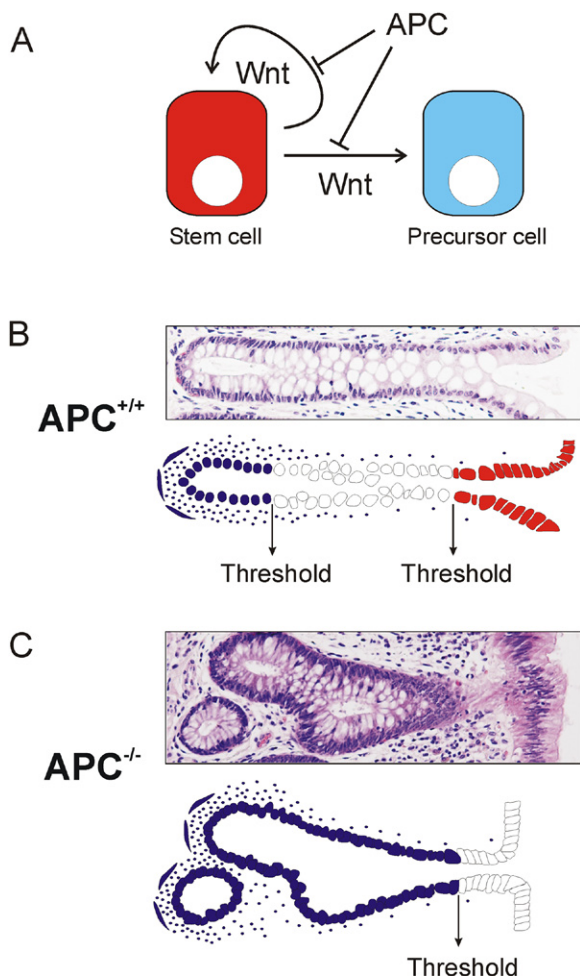
where certainty exists concerning the nature of the initiating mutation in the cancer. The importance of these mutations is underscored by the fact that these pathways are also mutated in sporadically occurring cases of the same cancers (Konishi et al., 1996; Luchtenborg et al., 2004). Even in cancers that are initiated by mutations in stability genes, the subsequent mutations in morphogenetic pathways might actually initiate neoplastic change by allowing clonal stem-cell growth. For example, Miyaki et al. found that WNT pathway-activating mutations are found in at least 65% of colorectal tumors of patients with HNPCC (Miyaki et al., 1999). In contrast to most oncogenes and tumor suppressor and stability genes, morphogens are not intrinsic signaling molecules, and they regulate not just one aspect of cell fate. Morphogens and the components of their intracellular signaling pathways (such as APC,  $\beta$ -catenin, SMAD4, PTEN, and RAS) are molecules involved in intercellular communication, and they couple many aspects of cell fate to the appropriate cellular position. We will therefore use the term *morphostats* in this review for morphogens and the components of their intracellular signal transduction networks with a role in tissue homeostasis in the adult. Of course, we realize that there is no strict division between molecules involved in intrinsic cellular signaling and those with a role in extrinsic signaling, and such a division is a simplification of biological reality. We feel, however, that such simplification can sometimes help to clarify concepts and advance our understanding of biology.

The concept of morphostasis holds that some of the factors that pattern a developing organism may be equally important in the maintenance of tissue homeostasis (morphostasis) after birth and thus be impor-

tant to prevent carcinogenesis. This concept was first advanced by Tarin (1972) and later further explored by Potter (2001), who introduced the term *morphostat*. One of the hallmarks of the development of colorectal cancer is that these *morphostats* are compromised because cell-fate determination is deregulated along the longitudinal axis as well as the axis of renewal at the earliest recognizable stages of colorectal carcinogenesis.

#### Adenoma to Carcinoma Sequence

It is now firmly established that most, if not all, colon carcinomas develop from a preceding noninvasive adenoma (glandular tumor; Figure 3) (Winawer et al., 1993). Adenomas are neoplasms that are defined by an expansion of the proliferating compartment and a lack of differentiation of epithelial cells that migrate toward the luminal surface of the crypts (dysplasia; Figure 3). Thus, the strict correlation between cellular phenotype and position along the vertical axis is disrupted in the adenoma. Substantial evidence exists to show that cell fate along the longitudinal axis of the gut is also disturbed in adenomatous polyps, as expression of a variety of markers of gastric epithelial differentiation has been described in colonic adenomatous polyps and colorectal carcinomas (Bara et al., 2003; Bartman et al., 1999; Kim et al., 2005; Koike et al., 2003; Smith and Watson, 2000). The earliest recognizable adenomatous lesion is the single-crypt adenoma, or dysplastic aberrant crypt focus (Nakamura and Kino, 1984; Preston et al., 2003; Takayama et al., 1998; Wright and Poulsom, 2002). The cells that form a single-crypt adenoma represent all the appropriate colonic epithelial-cell lineages and are of monoclonal origin, indicating that they originate from a single mutated cancer stem or precursor cell (Preston et al., 2003). Single-crypt adenomas



**Figure 4. Mutations in APC Lead to Deregulated Positional Signaling along the Axis of Renewal in the Colonic Crypt**

(A) WNT signaling determines a precursor-cell or stem-cell phenotype. APC plays an important role in the inhibition of WNT activity as cells migrate upwards away from the precursor- or stem-cell niche. (B) A model of WNT-regulated positional signaling in a normal colonic crypt. Note that this model is adapted from the classical model of morphogen signaling by Wolpert (1996) and a gross simplification of the reality in the colonic crypt. In reality, WNTs are lipid-modified molecules that are not freely diffusible, and multiple WNTs are expressed in both the epithelium and the mesenchyme of the colon. This model is based on the level of activity of the canonical WNT signaling pathway that can be estimated from the nuclear detection of  $\beta$ -catenin and expression of established WNT target genes. WNT (blue dots) is secreted in the precursor-cell niche; it forms a concentration gradient along the axis of renewal and has one or more threshold concentrations for colonic epithelial cells specifying different cell fate. At the highest threshold, WNT signaling specifies precursor-cell fate (blue cells). Functional APC is important for the interpretation of the threshold(s) and acts to diminish/shut down WNT signaling when a cell has traveled far enough from the precursor-cell niche to arrive at the first threshold concentration.

(C) If APC is mutated, cells are unable to shut down WNT signaling upon migration toward the level of the first threshold concentration, and they will therefore misinterpret the WNT gradient along the axis of renewal. As a result, these cells still perceive a level of WNT signaling that is above the highest threshold and maintain their precursor-cell phenotype. In this way, precursor or stem cells accumulate in the APC mutant crypt that subsequently expands through crypt fission.

initially expand through crypt fission, a process of crypt multiplication that occurs through budding and subsequent elongation to form two separate crypts (Figure 3) (Wasan et al., 1998; Wong et al., 2002). An elegant study by Greaves et al. studied colonic stem-cell behavior using the prevalence of nononcogenic mutations in the mitochondrial genome that result in detectable cytochrome c oxidase deficiency. With this method the authors were able to show that the progeny of a morphologically normal human colonic crypt stem cell that contains an mtDNA mutation can expand to occupy a whole crypt, and that this crypt then further expands by fission to form a patch of cytochrome c oxidase-deficient crypts (Greaves et al., 2006). These data indicate that crypt fission results from a symmetrical stem-cell division in which both daughter cells retain their stem-cell characteristics and each stem cell forms its own separate crypt. Crypts in the normal mucosa rarely show fission (Li et al., 1994). However, crypt fission rates are very high during intestinal growth and in situations of mucosal damage and repair (Cairnie and Millen, 1975; St Clair and Osborne, 1985). Apparently, the normal restraints on symmetrical stem-cell division are relieved under circumstances in which the number of stem cells needs to be augmented or replenished. Although the molecular mechanisms that control the rate of crypt fission are still poorly defined, it seems that WNT signaling is a positive regulator of the rate of crypt fission, as crypt fissioning is strongly increased in patients with FAP (see below). Just like stem cells in situations of damage and repair, adenomatous crypts seem to escape the normal restraints on symmetrical stem-cell division and show a high rate of crypt fission. In this way, the mutated stem cell in the adenomatous crypt has gained a competitive advantage over other stem cells in the same crypt and stem cells in neighboring crypts that allows clonal expansion of the adenoma. After the initial growth by crypt fission, the developing tumor later also expands by overgrowth of the adjacent normal crypts. In these overgrown crypts, adenomatous cells enter the crypt from the top, whereas the normally differentiated progeny of the residing nonmutated stem cell at the crypt base fills the lower end of the crypt (Preston et al., 2003). Cells in some of the larger adenomas progressively lose their differentiation and are able to grow through the basement membrane into the lamina propria, the intramucosal carcinoma stage. Ultimately, these tumors grow through the muscular layer that lies underneath the lamina propria and become an invasive carcinoma.

### Morphostats and Polyp Formation

Mutations in genes of the WNT, TGF- $\beta$  family, and receptor tyrosine kinase pathways have been identified as the germline mutation in inherited polyposis syndromes (Howe et al., 1998, 2001; Kinzler et al., 1991; Liaw et al., 1997; Marsh et al., 1998; Nishisho et al., 1991). Here we will focus on the most frequently mutated gene, APC, that is mutated in the FAP syndrome (Figure 4) (Kinzler et al., 1991; Nishisho et al., 1991). APC is a central reg-

ulator of the activity of the WNT pathway, and its mutation results in decreased breakdown and increased nuclear translocation of  $\beta$ -catenin, the effector protein of the WNT pathway (Gregorieff and Clevers, 2005). Although APC is often classified as a tumor suppressor gene, its role extends beyond a role in cell-cycle arrest or apoptosis. APC plays a central role in the transduction of extrinsic WNT signals to the intestinal epithelial stem-cell niche. WNT signaling induces a coordinate response in the intestinal epithelial transcriptome that specifies multiple aspects of precursor-cell fate (van de Wetering et al., 2002). For example, WNT signaling is a master switch in precursor-cell proliferation and negatively controls the expression of intestinal epithelial differentiation markers (van de Wetering et al., 2002). Furthermore, WNT signaling regulates precursor-cell positioning via the EphB/Ephrin-B receptor-ligand system (Battile et al., 2002). The broad role for APC in the regulation of intestinal epithelial-cell fate has been made very clear by elegant experiments performed by Sansom et al. (2004). The authors showed that conditional deletion of APC resulted in rapid nuclear accumulation of  $\beta$ -catenin and gross disturbance of epithelial homeostasis. Acute APC deficiency resulted in expansion of the precursor-cell population by increased precursor-cell proliferation and abrogation of their migration along the crypt-villus axis. Cellular positioning was deregulated, as proliferation occurred independently of cellular position and Paneth cells were distributed throughout the expanded crypt-like area, in contrast to their normal position at the crypt base. Loss of APC altered cell-fate specification with a loss of goblet cells, reduced numbers of endocrine cells, and loss of expression of epithelial differentiation markers.

From the histopathological model of adenoma growth summarized above, it would follow that any initiator of the adenoma carcinoma sequence should also disrupt the normal constraints on the rate of crypt fission. Indeed, crypt fission in the normal mucosa of FAP patients (who are heterozygous for the APC mutation) is increased 19-fold compared to the rate of crypt fission in the mucosa of unaffected control patients (Wasan et al., 1998). Increased nuclear localization of  $\beta$ -catenin is observed specifically in crypts that are in the process of fission (Preston et al., 2003). These data suggest that APC acts as a negative regulator of the rate of crypt fission by inhibition of the WNT pathway at the level of  $\beta$ -catenin. The fact that mutations in  $\beta$ -catenin that make  $\beta$ -catenin resistant to proteolytic degradation are also found in colorectal cancers and the fact that such mutant  $\beta$ -catenin leads to polyposis in mice indicate that the results of the APC mutation are mainly due to its role in the canonical WNT signaling pathway (Harada et al., 1999). Therefore, appropriate inhibition of canonical WNT signaling not only acts to restrict the precursor-cell compartment to the precursor-cell niche within a single colonic crypt but also functions as an important brake on lateral stem-cell expansion through crypt fission

and in this fashion inhibits clonal cell growth. Given the important role of APC in WNT signaling and the correct interpretation of extracellular WNT signals, it appears to be legitimate to consider APC in the first place as a morphostat.

### Conclusion

The phenotype of intestinal epithelial cells is patterned along two different axes. The same morphogenetic molecules that specify positional information in a developing embryo specify cell fate along the vertical axis in the adult, and these may also specify cell fate along the longitudinal axis, although this is still less well established. The link between position and function of epithelial cells in a crypt is disrupted at the earliest recognizable stage of colorectal carcinogenesis. This corresponds with the finding that mutations that are capable of *initiating* clonal cell growth in the colon such as those in APC are mutations in morphogenetic pathways that compromise position-dependent regulation of multiple aspects of cellular fate. Since morphogenetic pathways are responsible for the maintenance of the stability and balance of the mucosal microarchitecture in the adult, the term "*morphostats*" might aptly categorize these genes, in analogy with their role as morphogens during development.

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